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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS AND ESSENTIAL OIL OF ROOTS OF *CIPADESSA BACCIFERA* (ROTH.) MIQ

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ABSTRACT

In the present study, we aimed to evaluate the antimicrobial activity of solvent extracts and essential oil of roots of Cipadessa baccifera (Roth.) Miq. The plant samples for investigation were collected from Thavarekere and Savandurga, Magadi Taluk, Bengaluru Rural district, and outskirts of Bengaluru, and identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971. The dried root samples were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses. The root samples were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water. The essential oil from root sample was extracted in Clevenger apparatus. The sequential extracts and essential oil of roots of C. baccifera (Roth) Miq. were subjected to evaluation of antimicrobial activity against diarrhoea, skin, wound and oral infections causing selected pathogens, including 07 Gram positive, 06 Gram negative bacteria and 06 fungal strains. Results revealed that the broad spectrum of anti-bacterial activity of roots of C. baccifera revealed in the present investigation gives scientific validity for its usage in treatment of dysentery, skin related disorders and wounds in traditional medicines.

Keywords: Cipadessa Baccifera, Roots, Antibacterial, Antifungal, Essential Oil.

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Original Article

INTRODUCTION

Infectious diseases caused by microorganisms is one of the leading causes of mortality worldwide which is a nagging challenge and is of great concern to the scientific community even to this day. Microorganisms are one of the oldest of creatures on this planet to have successfully evolved, adapted and survived all the vagaries of nature since millions of years[1]. Even today traditional medicines used for treatment of infectious maladies include scores of plants like, barberry (*Arctostaphylos uva-ursi)* and cranberry juice (*Vaccinium macrocarpon)* for urinary tract infections, while lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are used as broad-spectrum antimicrobial agents [2].

The discovery of antibiotics no doubt revolutionized medicine, drastically bringing down the morbidity and mortality rates due to infectious diseases. However, it led to rampant misuse, indiscriminate or inappropriate use of commercial antibiotics. This resulted in the development of antibiotic resistance in bacterial pathogens against many microbial infections, an alarming phenomenon that has serious public health concern with economic and social implications [3]. As a consequence, the choices of antibiotic treatment against the already existing or multidrug resistant bacterial infections are becoming limited, resulting in high morbidity and increased mortality

rates[4]. The prevalence of many highly resistant clinical isolates such as, *Staphylococcus aureus, Streptococcus pyogenes, Mycobacterium tuberculosis, Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae* etc. have been reported in the last few decades [1].

Essential oils (EOs) are aromatic volatile liquids secreted by oil secreting cells, glandular hairs or secretion ducts in different parts of the plants and stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes. They are biosynthesized in plants through secondary metabolism giving it a characteristic odour, flavour and different colours ranging in shades of pale yellow to emerald green and sometimes even blue to dark brownish red [5,6], Essential oils are natural, complex hydrophobic mixtures containing from a dozen to several hundred components. The major components identified primarily include terpenes and terpenoids. Terpenes are made of different combinations of 5 carbon isoprene units and are classified into monoterpenes, sesquiterpenes, diterpene, and triterpene [7,8]. A variety of other compounds found in the essential oils may include saturated and unsaturated hydrocarbons, aldehydes, esters, ethers, ketones, oxides, phenols, alcohols, sulphur and nitrogen containing compounds, coumarins and homologues of phenylpropanoids. The antimicrobial property of EOs known for centuries has found important applications today in diverse commercial products such as dental root canal sealer [9], antiseptics[10], show significant antifungal [11], antiviral [12], and antioxidant activities [13].

Considerable number of studies conducted on the antimicrobial activity of medicinal plants indicates that they are a promising source of potent antimicrobials which include secondary metabolites such as saponins, tannins, phenols, alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and esters. Hence plants have been successfully used worldwide in traditional medicines to treat several diseases and infections [14]. Evaluation of the antimicrobial potency of ethnomedicinal plants such as *Cipadessa baccifera* which has been widely used in the treatment of dysentery, skin and wound infections etc… is relevant in this context[15]. With this scenario, the present study was designed to conduct with the main purpose of evaluation of antimicrobial activity of solvent extracts and essential oil of roots of *C. baccifera* (Roth.) Miq.

MATERIALS AND METHODS

Collection of Plant Material

The plant samples for investigation were collected from Thavarekere and Savandurga, Magadi Taluk, Bengaluru Rural district, and outskirts of Bengaluru. The plant under study was identified as *C. baccifera* (Roth) Miq. as per Flora of Hassan (1976) and Flora of Karnataka (1996) by Saldana [16,17]. Further, the identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971.

Sample Processing

The samples such as roots of *C. baccifera* were collected in clean and sterile polythene bags for various analyses. The collected samples were washed thoroughly in running tap water to remove dust and soil particles and were blotted dry. Healthy and infection free roots were separated and shade dried for 20 days. The dried roots were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

Sequential Extraction

Dry and coarsely powered roots of *C. baccifera* were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water[18]. Then the solvents were

filtered and concentrated to dryness under pressure using rotary vacuum evaporator. The root extracts were air dried to remove the solvents completely, then sealed and stored at 4°C in a refrigerator for further studies.

Essential Oil Extraction

About 100 g each of the powered root samples was subjected to hydrodistillation for 10 hours in a Clevenger apparatus [19]. The extracted oil samples were collected by solubilizing in hexane. Hexane was then allowed to evaporate completely at room temperature. The process of hydrodistillation extraction was repeated several times; the oil obtained was pooled and stored in vials at 4ºC in a refrigerator for further analyses.

ANTIMICROBIAL ACTIVITY

Test Microorganisms

The sequential extracts and the essential oil from roots of *C. baccifera* were evaluated for their antimicrobial activity against selected pathogens causing diarrhoea, skin, wound and oral infections. The diarrhoea causing pathogens include, Gram positive bacteria, *Bacillus cereus* NCIM 2155, Gram negative bacteria *viz.*, *Escherichia coli* NCIM 2343, *Shigella flexneri* NCIM 5265 and *Salmonella abony* NCTC 5080. The skin and wound infections causing pathogens include Gram positive *Propionibacterium acnes* ATCC 11827, *Nocardia asteroides* MTCC 274 and *Staphylococcus aureus* MTCC 96 and Gram negative *Pseudomonas cepacia* NCIM 5089, *Pseudomonas aeruginosa* MTCC 741 and *Candida* sp. such as, *Candida krusei* MTCC 9215 and *Candida parapsilosis* MTCC 6510. The pathogens causing oral infections selected were Gram positive *Streptococcus gordonii* MTCC 2695, *Streptococcus mutans* MTCC 497 and *Corynebacterium diphtheriae* NCIM 5212 and fungal sp., *Candida albicans* ATCC 10231, *Candida glabrata* MTCC 3019 and *Fusarium* NCIM 894. In addition, antimicrobial activity was evaluated against Gram negative, *Klebsiella pneumoniae* NCIM 2719 and fungal strain, *Aspergillus niger* NCIM 501. These microorganisms were procured from American Type Culture Collection (ATCC), National Collection of Industrial Microorganisms (NCIM), National Culture of Type Cultures (NCTC) and Microbial Type Culture Collection (MTCC) Institutes.

Determination of Zone of Inhibition (ZOI)

The standard protocols of Clinical and Laboratory Standards Institute (CLSI) and National Committee for Clinical Laboratory Standards (NCCLS) for screening of antimicrobial activity of the sequential extracts and essential oils of roots of *C. baccifera* by agar well diffusion method were followed. The stock solution concentration of 10 mg/mL of solvent extracts and essential oils were prepared in DMSO. The stock concentration of 1 mg/mL of antibiotics Ciprofloxacin and Ketoconazole were prepared and used as positive controls for bacteria and fungi respectively. The test was carried out in triplicate [20, 21].

Further, based on the zone diameter the antimicrobial activity of standard antibiotic ciprofloxacin against bacteria was expressed as resistant (ZOI is ≤ 15 mm), intermediate (ZOI is between 16-20 mm) and sensitive/susceptible (ZOI is ≥ 21 mm) and for Ketoconazole against fungi was expressed as resistant (ZOI is ≤ 22 mm), intermediate (ZOI is between 23-29 mm) and sensitive/susceptible (ZOI is ≥ 30 mm) [20,21]. The sensitivities of the microorganism species to the plant extracts were determined by measuring the size of inhibitory zones (including the diameter of well) on the agar surface and values <8 mm were considered as not active against microorganisms.

Minimum Inhibitory Concentration (MIC) Assay

Minimum inhibitory concentration (MIC) was determined by modified resazurin assay using microtiter-plate technique described by Sarker [22].Each plate had a set of controls; the column with positive control contained the broad spectrum antibiotics Ciprofloxacin for bacteria and Ketoconazole for fungi, whilst the negative control column had all solutions except test extracts and sterility control that is, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of nutrient broth instead. The plates were incubated for 18 to 24 hours at 37°C at 100% relative humidity. The change in colour of resazurin dye was observed and assessed visually. Any change in colour from purple to pink to colourless was recorded as positive result. The lowest concentration prior to which the positive colour change occurred was taken as the MIC value for that particular test sample against the tested bacteria and fungi. The average of three values was taken to be the MIC of the test sample and the bacterial/fungal strain.

RESULTS AND DISCUSSION

Antimicrobial Activity of Sequential Root Extracts of *C. Baccifera*

The antimicrobial activity of the sequential hexane, chloroform, methanol and aqueous extracts of roots of C. baccifera was assessed and the results are presented in Tables 1. The results revealed that the anti-bacterial activity of hexane extract of root of *C. baccifera* against *Bacillus cereus* showed the highest inhibition zone of 20 mm, while intermediate zones of inhibition were observed for *Propionibacterium acnes* (18 mm), *Escherichia coli* (17 mm), *Pseudomonas cepacia* (17 mm) and *Streptococcus gordonii* (16 mm). The chloroform extract of root exhibited anti-bacterial activity against *Bacillus cereus, Shigella flexneri, Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Streptococcus gordonii* and *Streptococcus mutans*, with the zones of inhibition in the intermediate range of 15 to17 mm. The methanolic extract exerted maximum inhibition of *Escherichia coli* (20 mm) and *Streptococcus gordonii* (20 mm), while *Shigella flexneri* (19 mm), *Pseudomonas aeruginosa* (17 mm) and *Bacillus cereus* (16 mm) were effectively inhibited with comparatively smaller zones of inhibition. In the aqueous extract of the root, *Escherichia coli*, *Shigella flexneri*, *Bacillus cereus, Propionibacterium acnes*, *Pseudomonas aeruginosa*, were inhibited with intermediate zones of inhibition in the range. The highest zone of inhibition of 16 mm was obtained for *Candida albicans* in hexane extract of the root. However, the anti-fungal activity of rest of the extracts of root was not found to be significant. Among the pathogens tested significant anti-bacterial activity of the root extracts was observed against *Escherichia coli, Bacillus cereus and Streptococcus gordonii*.

Table 1: Antimicrobial Activity of Sequential Extracts of Root Extracts of *C. Baccifera*

Microorganisms	Std.	Zone of inhibition (mm) Solvent extracts of root			
		HE	CE	ME	AE
Causative agents of diarrhoea					
Escherichia coli	16±0.35	17±0.11	14±0.64	20±0.25	19±0.43
Shigella flexneri	30±0.22	14±0.82	15±0.64	19±0.81	17±0.43
Bacillus cereus	34±0.32	20±0.62	17±0.93	16±0.61	16±0.68
Causative agents of skin and wound infections					
Propionibacterium acnes	14±0.67	18±0.81	15±0.16	19±0.32	17±0.65
Pseudomonas aeruginosa	14±0.76	13±0.47	15±1.07	17±0.12	16±0.71
Pseudomonas cepacia	14±0.7	17±0.32	15±0.5	14±0.39	13±0.33
Causative agents of oral infections					
Streptococcus gordonii	15±0.2	16±0.21	17±0.43	20±0.32	15±0.12
Streptococcus mutans	14±1.21	14±0.91	17±0.15	13±0.26	14±0.34

Table 1: Contd.,

Corynebacterium diphtheriae	15±1.34	15±1.36	14±1.15	14±033	12±0.38
Candida albicans	12±0.38	16±0.64	11±0.66	14±0.82	13±0.33
Candida glabrata	-	-	-	11±0.82	-
Fusarium	-	-	-	11±0.77	-

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Choloform Extract; ME - Methanol Extract; AE - Aqueous Extract; -: ZOI <10mm *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus sp.* were not inhibited

Minimum Inhibition Concentration (MIC) Root Extracts of *C. Baccifera*

Significant anti-bacterial activity was observed against *Escherichia coli*, in methanolic and hexane extracts of the root at MIC of 62.5 µg/mL and 125 µg/mL respectively. *Shigella flexneri* was inhibited in MIC of 250 µg/mL of hexane, methanol and aqueous extracts of root. While MIC of 250 µg/mL of chloroform and hexane extracts of root effectively inhibited *Bacillus cereus.* The hexane, methanol and aqueous extracts of root exhibited the least MIC of 125 µg/mL for *Propionibacterium acnes*. The growth of *Pseudomonas aeruginosa* was inhibited in 250 µg/mL MIC of chloroform extract. While for *Pseudomonas cepacia* the least MIC of 62.5 µg/mL was obtained in the hexane extract of the root. *Streptococcus gordonii* was found to be susceptible in MIC of 250 µg/mL of chloroform, methanol and aqueous extracts. The least MIC of 125 µg/mL was observed in chloroform extract for *Streptococcus mutans*. The hexane, chloroform and methanol extracts of root showed potent anti-bacterial activity against *Corynebacterium diphtheria* at MIC of 250 µg/mL.

Significant anti-fungal activity of hexane extract of root was observed against *Candida albicans* (MIC of 125 µg/mL). The higher MIC value of 1000 µg/mL of the different solvent extracts of root indicated no significant activity against fungal strains, *Candida glabrata* and Fusarium. Both zone of inhibition and minimum inhibitory concentrations were found to be non-significant in the solvent extracts of *Cipadessa baccifera* against some of the bacterial strains such as, *Salmonella abony, Klebsiella pneumoniae, Nocardia asteroides, Staphylococcus aureus* and fungi such as *Candida krusei, Candida parapsilosis* and *Aspergillus niger*.

Table 2: Minimum Inhibitory Concentration (MIC) of Sequential Extracts of Root of *C. Baccifera*

Microorganisms	Std.	MIC (µg/mL)			
		Solvent extracts of root			
		HE	CE	ME	AE
Causative agents of diarrhoea					
Escherichia coli	62.5	125	250	62.5	125
Shigella flexneri	62.5	500	250	250	250
Bacillus cereus	15.62	250	250	500	500
Causative agents of skin and wound infections					
Propionibacterium acnes	500	125	250	125	125
Pseudomonas aeruginosa	31.25	500	250	250	250
Pseudomonas cepacia	15.62	62.5	125	500	500
Causative agents of oral infections					
Streptococcus gordonii	7.81	500	250	250	250
Streptococcus mutans	62.5	250	125	500	500
Corynebacterium diphtheriae	1000	250	250	250	500
Candida albicans	31.25	125	1000	1000	1000
Candida glabrata	15.62	1000	1000	1000	1000
Fusarium	0.97	1000	1000	1000	1000

Mean ± SD; Std-Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Choloform Extract; ME - Methanol Extract; AE - Aqueous Extract; -: MIC > 1000 µg/mL *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus* were not inhibited

Antimicrobial Activity of Essential Oils of Roots of *C. Baccifera*

The essential oil of root of *C. baccifera* showed potent anti-bacterial activity against *Escherichia coli* with highest zone of inhibition of 21 mm and least MIC of 62.5 µg/mL (Table 4). *Bacillus cereus*, *Shigella flexneri* and *Salmonella abony* were inhibited in the root oil with intermediate zones of inhibition in the range of 15-18 mm and MIC of 250 µg/mL. *Pseudomonas cepacia* was found to be susceptible to the root essential oil with an inhibition zone of 18 mm and MIC of 125 µg/mL (Table 4). The zones of inhibition of *Propionibacterium acnes*, *Pseudomonas aeruginosa* and *Nocardia asteroides* were found to be smaller and MIC value less significant. An intermediate zone of 17 mm and MIC of 250 µg/mL was obtained in root oil for *Streptococcus gordonii.* However, *Streptococcus mutans* and *Corynebacterium diphtheriae* were susceptible to the root oil at higher MIC. The anti-fungal activity of the root oil was found to be not significant (Table 3).

Table 3: Zone of Inhibition of Leaf Oils of *C. Baccifera*

Microorganisms	Std.	ZOI (mm) Root oil
Causative agents of diarrhoea		
Escherichia coli	16±0.35	21±0.43
Shigella flexneri	30±0.22	15±0.20
Bacillus cereus	34±0.32	16±0.2
Salmonella abony	35±1.22	18±0.21
Causative agents of skin and wound infections		
Propionibacterium acnes	27±0.60	15±0.12
Pseudomonas cepacia	23±0.71	18±0.5
Pseudomonas aeruginosa	37±0.56	10±0.23
Nocardia asteroides	39±0.83	11±0.21
Causative agents of oral infections		
Streptococcus gordonii	35±0.13	17±0.02
Streptococcus mutans	36±0.52	11±0.13
Corynebacterium diphtheriae	32±0.51	13±0.34
Candida albicans	26±0.43	11 ±0.42
Candida glabrata	14±0.31	-
Fusarium	13±0.22	16 ±0.11

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi; -: ZOI <10 mm *S. aureus, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus niger* were not inhibited

MIC of Essential Oils of Roots of *C. Baccifera*

The MIC of root oils of *Cipadessa baccifera* for the tested microorganisms ranged from 62.5 to over 1000 µg/mL (Table 4). The MIC values for the essential oils for the filamentous fungi indicate that they were not effectively inhibitory against the fungal strains at low concentration. However, among the *Candida* species, *Candida glabrata* was most sensitive, while rest of the strains was not inhibited by the essential oils.

Table 4: Minimum Inhibitory Concentration (MIC) of Essential Oil of Roots of *C. Baccifera*

Microorganisms	Std.	MIC (µg/mL)
		Root oil
Causative agents of diarrhoea		
Escherichia coli	62.5	62.5
Shigella flexneri	62.5	250
Bacillus cereus	15.62	250
Salmonella abony	500	250
Causative agents of skin and wound infections		
Propionibacterium acnes	500	250
Pseudomonas cepacia	31.25	125
Pseudomonas aeruginosa	15.62	1000
Nocardia asteroides	31.25	500
Causative agents of oral infections		
Streptococcus gordonii	7.81	250
Streptococcus mutans	62.5	500
Corynebacterium diphtheriae	1000	250
Candida albicans	31.25	500
Candida glabrata	15.62	1000
Fusarium	0.97	1000

Mean ± SD; Std-Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Choloform Extract; ME - Methanol Extract; AE - Aqueous Extract; -: MIC > 1000 µg/mL *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus* were not inhibited

Natural plant based antimicrobial compounds have enormous therapeutic potential as they do not cause side effects which are often associated with synthetic antimicrobials. The hexane, chloroform, methanol &aqueous extracts, and essential oils of roots of *C. baccifera* (Roth) Miq. were subjected to evaluation of antimicrobial activity against diarrhoea, skin, wound and oral infections causing selected pathogens, including 07 Gram positive, 06 Gram negative bacteria and 06 fungal strains. Previous studies have shown that antimicrobial potential could be due to the presence and distribution of phytochemicals such as flavonoids, phenolic compounds, tannins, coumarins, saponins and alkaloids [23].

The results of antimicrobial activities of root extracts of *C. baccifera* revealed that root extract exhibited considerable antimicrobial activities. The total phenolic and flavonoid content in the root along with presence of alkaloids, saponins and tannins could explain the strong antimicrobial potential of the root. As reported byBriskin[24], the combination of some of these phytochemicals could be responsible for the observed antimicrobial potential of the various solvent extracts.

Considerable variation was observed in the degree of antimicrobial activity of the hexane, chloroform, methanol and aqueous solvent extracts of root of *C. baccifera.* This indicates that bioactive-antimicrobial molecule in root may be both polar and non-polar in nature. In the root, both hexane and methanolic extracts exhibited higher antimicrobial activity. The variation in antimicrobial activity in different solvent extracts of root of *C. baccifera* could be attributed to the polar, non-polar nature of the bioactive compounds, insolubility or difference in degree of solubility of phyto constituents in different solvents and their denaturation during extraction process [25,26].

Some of bacteria which were effectively inhibited by the solvent extracts of *C. baccifera* in this study include, some common pathogens causing diarrhoea such as, *Escherichia coli, Shigella flexneri* and *Bacillus cereus*. Among the skin and wound infection causing pathogens, *Propionibacterium acnes*, *Pseudomonas cepacia* and *Pseudomonas aeruginosa* were effectively inhibited, however no significant inhibitory effect was observed against *Nocardia asteroides*, *Staphylococcus aureus, Candida parapsilosis* and *Candida krusei.* The causative agents of oral infection *viz.*,

Streptococcus gordonii, *Streptococcus mutans*, *Corynebacterium diphtheriae*, *Candida albicans*, *Candida glabrata* and *Fusarium* were also effectively inhibited by the root extracts of *C. baccifera*. These findings are consistent with results of previous studies on *C. baccifera* and other species of Meliaceae [27-29].

The antimicrobial study results clearly indicate that the anti-bacterial activity was found to be more pronounced against the Gram positive bacteria followed by Gram negative bacteria and fungi. Five among the seven selected Gram positive pathogens, four of the six Gram negative and two of the six fungal pathogens were found to be effectively inhibited. Similar observations were reported by Thiruvanukarasu *et al.*, where Gram positive bacteria were inhibited effectively when compared to Gram negative and fungal pathogens by *C. baccifera*[29]. This difference in sensitivity of the Gram positive and negative bacteria to the solvent extracts could be attributed to the inherent structural difference in their cell walls. The Gram negative bacteria possess an outer phospholipid membrane carrying the lipopolysaccharide component, which acts as a barrier to many antimicrobial agents including antibiotics due to its intrinsic nature of impermeability. However, the Gram positive bacteria are more susceptible due to its peptidoglycan cell wall which is not an effective permeability barrier [30]. In the present study significant anti-fungal activity was observed only against *Candida albicans* and *Fusarium* species. However, inhibition of *C. krusei*, *C. parapsilosis* and *Aspergillus niger* was not significant. *C. albicans* was less sensitive to plant extracts compared to Gram positive and Gram negative bacteria. This difference in susceptibility between eukaryotic cells of *C. albicans* and *Fusarium* and the prokaryotic cells of bacteria may be attributed to their difference in cell type which is in accordance with findings of antimicrobial studies carried out by Oskay and Sari, and Obeidat *et al.*[31,32].

Antimicrobial potential of essential oils derived from plants is the basis of many applications especially in food preservation, aromatherapy and medicine [25]. The essential oils of roots of *Cipadessa baccifera* were found to show varied degree of inhibition on the tested microorganisms. The antimicrobial study of essential oils of *C. baccifera* showed effective and broad spectrum antimicrobial activity wherein, the essential oil of root exhibited the moderate antimicrobial activity. Sesquiterpenes especially caryophyllenes are known to possess anti-inflammatory, anti-bacterial, anti-fungal and spasmolytic properties [33]. The antimicrobial activity in the root oil of *C. baccifera* could be attributed principally to the presence of a significant amount of 17.32% of sesquiterpene *viz.*, caryophyllene. The root oil exhibited significant antibacterial activity against *Escherichia coli*. These findings were in accordance with previous research investigations reported in the literature[34,35].

The antimicrobial activity observed in the present study could possibly be explained by two modes of action. Firstly, the essential oil may disrupt the bacterial cell membrane resulting in the loss of ions, changes in membrane potential, disturbance of the proton pump, leading to the lysis of the bacteria. The second mechanism could involve the inhibition of production of amylase and protease which stops the toxin production, electron flow, thereby resulting in coagulation of the cell content as reported by Nazzaro [36]. Ultimately these events lead to bacterial cell death [10]. The anti-fungal activity of the oils was potent only against *Candida glabrata*, while the inhibition of other fungi was not significant. The mechanism of anti-fungal effect of essential oils is similar to bacteria as reported in earlier researches [5,37.]

CONCLUSION

In conclusion, the broad spectrum of anti-bacterial activity of roots of *C. baccifera* revealed in the present investigation gives scientific validity for its usage in treatment of dysentery, skin related disorders and wounds in traditional medicines.

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